6-Hydroxyflavones and Other Flavonoids of *Crocus*

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6-Hydroxyflavones have been identified for the first time in the Iridaceae, in leaves of three *Crocus* species. Three new glycosides have been characterised: 6-hydroxyluteolin 7-rhamnosyl-glucoside, scutellarein 7-glucoside and scutellarein 7-methyl ether 6-glucoside, as well as two known glycosides: 6-hydroxyluteolin 7-glucoside and 6-hydroxyluteolin 7-methyl ether 6-glucoside. 6-Hydroxyluteolin and scutellarein glycosides have been found before in Bromeliaceae, Commelinaceae, Cyperaceae and Orchidaceae, but this is the first record of the respective 7-methyl ethers in the Monocotyledoneae. Acacetin and tricin have been identified as aglycones in *C. laevigatus* and *C. heuffelianus* leaves, respectively and the occurrence of mangiferin confirmed in *C. aureus* leaves. Two of the major flavonol glycosides present in flowers of cultivated species were identified as kaempferol 3-sophoroside and kaempferol 3-rutinoside-7-glucoside. However none of the flavonoids identified appears to contribute to yellow petal colour in *Crocus*, which is probably entirely carotenoid-based.

Introduction

As part of a continuing chemotaxonomic survey of flavonoids in families of the Monocotyledoneae [see *e.g.* 1, 2], our attention has turned to the Iridaceae, which contains some 60 genera and 800 species. Apart from the genus *Iris*, the family has not been extensively investigated and the results of a general flavonoid survey will be presented later. This paper describes the identification of some of the major flavonoids found in the genus *Crocus*. In spite of its ornamental importance, this genus has not yet been studied in detail for its phenolic constituents. *Crocus* is mainly known as a source of the unusual water-soluble carotenoid crocetin and its digentiobiose ester, crocin, which has been used commercially as a yellow food colourant. Crocin is usually obtained from the stigmas of the meadow saffron *C. sativus*, but also is recorded in yellow petals of *C. albiflorus* and *C. luteus* [3].

From the viewpoint of flavonoid chemistry, the only major survey of *Crocus* has been that of Bate-Smith [4] who found glycoflavones and the two flavonols kaempferol and quercetin to be widespread in hydrolysed leaf extracts of 49 spp. Among rarer phenolic constituents provisionally identified in his survey were tricin (in 3 spp.), myricetin (in 4 spp.) and the glucoxanthone mangiferin (in 2 spp.). There was little correlation between the distribution of these compounds and the classification of the genus according to Maw [5], but some evidence was obtained of a relationship between chemistry and geography, especially among the Eastern and Western Mediterranean species [6].

Two of the main reasons why the flavonoids of *Crocus* have not been better investigated is the limited amount of plant material available for most taxa and the difficulty of taxonomic verification. A wild species collection is being developed at the Royal Botanic Gardens, Kew [7] and it is hoped eventually to screen these plants for flavonoids. The present work has perforce been carried out on those species and cultivars, which are grown commercially and hence are available in quantity. Our aim here has been to establish the nature of the main kinds of flavonoid of leaf and petal and we here report the identification of the major constituents that we have encountered. Some of these results have been briefly mentioned in an earlier publication [6].

Results

The first leaf flavones to be identified were not detected by Bate-Smith [4] in his survey, but were recognised in a parallel survey [6] carried out at the same time to be unusual in structure, being visibly yellow in daylight. They occurred in leaves of *C. chrysanthus* cv. Cream Beauty, *C. corsicus* and *C. minimus* and have now been fully characterised

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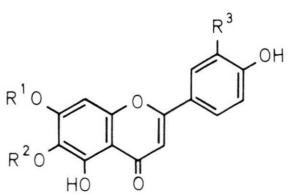
Table I. 6-Hydroxyflavone glycosides of *Crocus* leaves.

Glycoside	Source(s)
6-Hydroxyluteolin 7-glucoside (1)	*C. chrysanthus* cv. Cream Beauty
6-Hydroxyluteolin 7-rhamnosylglucoside (2)	*C. minimus*
6-Hydroxyluteolin 7-methyl ether 6-glucoside (3)	*C. minimus, C. corsicus*
Scutellarein 7-glucoside (4)	*C. chrysanthus* cv. Cream Beauty
Scutellarein 7-methyl ether 6-glucoside (5)	*C. minimus, C. corsicus*

as five 6-hydroxyflavone glycosides, as shown in Table I.

Identification was by standard procedures, involving in all cases comparison at some stage with authenic pigments. Three of the glycosides are new: 6-hydroxyluteolin 7-rhamnosylglucoside (**2**), scutellarein 7-glucoside (**4**) and scutellarein 7-methyl ether 6-glucoside (**5**); their structural analyses presented no particular difficulties (see Experimental). The fourth compound, 6-hydroxyluteolin 7-methyl ether 6-glucoside (**3**) has been described before, from *Sesamum indicum* (Pedaliaceae) [8], but it is quite rare. Since no standard was available, the identity of the aglucone, namely 6-hydroxyluteolin 7-methyl ether, was confirmed by mass spectral (MS) analysis and by its demethylation to 6-hydroxyluteolin; location of the methyl at the 7-position followed from UV-spectral analysis and the MS fragmentation pattern. The remaining glycoside, 6-hydroxyluteolin 7-glucoside (**1**) has been reported before from several sources [9].

The two 6-hydroxyflavone glycosides with 7-O-methylation, *i.e.* **3** and **5**, occur together in *C. corsicus* and *C. minimus*, two species which are in the same section *Involucrati* of the genus. It is interesting that although they differ in corm-tunic characters, they are otherwise fairly close to each other morphologically and both are native to the island of Corsica. By contrast 6-hydroxyflavone glycosides without 7-O-methylation are restricted to one species, *C. chrysanthus*, which is in a different section from the first two species, namely in *Annulati*.



1: R^1 = Glc, R^2 = H, R^3 = OH
2: R^1 = Glc-O-Rha, R^2 = H, R^3 = OH
3: R^1 = Me, R^2 = Glc, R^3 = OH
4: R^1 = Glc, R^2 = R^3 = H
5: R^1 = Me, R^2 = Glc, R^3 = H

According to Bate-Smith [4], ordinary flavone glycosides are relatively uncommon in leaves of *Crocus*, luteolin being provisionally noted by him in hydrolyzed extracts of one species (*C. nevadensis*) and tricin in hydrolysed extracts of three (*C. korolkowii, C. graveolens* and *C. hyemalis*). We could not confirm tricin in our material of *C. korolkowii* but were able to find it in another wild species, *C. heuffelianus;* its identity was confirmed by cochromatography with authentic material. We also found small amounts of an apigenin glycoside, along with the 6-hydroxyflavones, in *C. minimus* leaf. Another common flavone aglycone, not recorded before [4], was found in leaves of *C. laevigatus* and identified as acacetin (apigenin 4′-methyl ether). In summary, then, the flavone O-glycoside profile of *Crocus* includes, in addition to the 6-hydroxyflavones (Table 1), derivatives of apigenin, acacetin, luteolin and tricin but none of these derivatives is widely present in the genus.

The major flavone type in *Crocus* leaves, according to the earlier survey [4], is C-glycosylflavone, found in 23 of 49 species studied. There were other flavonoids in the nine species we examined in detail (Table II) and luteolin-based glycoflavones were noted specifically in *C. corsicus* and *C. minimus*. Confirmation that glycoflavones occur in *Crocus* has been obtained in the case of *C. reticulatus*, from which Sergeyeva [10] has isolated a scoparin O-rhamnosylglucoside. In addition, the C-glucoxanthone, mangiferin, is well known to occur occasionally in higher plant groups in the same context as glycoflavones and Bate-Smith in his survey recorded mangiferin (from its colour reactions) to be present in *C. aureus* and in *C. stellaris* (a possible hybrid plant involving *C. aureus*) [4]. We

Table II. Flavonoid constituents of petals and leaves of cultivated *Crocus* species.

Species and petal colour	Petal constituents				Leaf constituents		
	Dp glyc.	Crocetin deriv.	Km soph.	Km 3RG7G	6 OH flav.	Km soph.	other
C. aureus Sibth. & Sm. (yellow)	–	+	+	–	–	–	mangiferin
C. chrysanthus Herb. cvs. (various)	(+)	(+)	–	+	+	–	–
C. corsicus Maw (lilac)	+	–	+	–	+	+	–
C. etruscus Parl. (lilac)	+	+	+	+	–	+	–
C. fleischeri Gay (white)	–	–	–	+	–	–	–
C. korolkowii Maw & Regel (yellow with purple)	+	+	+	–	–	+	–
C. laevigatus Bory & Chaub (white)	–	+	+	–	–	+	acacetin glyc.
C. minimus DC. (lilac)	+	–	+	+	+	–	apigenin glyc.
C. versicolor Ker-Gawl (white)	–	–	+	–	–	+	–

Key: Dp glyc. = delphinidin 3,5-diglucoside in *C. laevigatus*; unidentified glycoside in other spp., Km soph. = kaempferol 3-sophoroside; Km 3RG7G = kampferol 3-rutinoside-7-glucoside, 6 OH flav. = 6-hydroxyflavones (Table I for details). Additionally, quercetin was detected as an aglycone in petals of all spp. except *C. chrysanthus* and *C. fleischeri*.

have now been able to confirm mangiferin in *C. aureus* leaves by its isolation in crystalline form.

The only earlier reports of flavonols in *Crocus* were those of Price *et al.* [11], who isolated free kaempferol from petals of two yellow-flowered species, *C. asturicus* and *C. speciosus*, and Kuhn and Low [12] who obtained isorhamnetin 3,4′-diglucoside from pollen of the cultivar 'Sir John Bright'. Bate-Smith in his leaf survey [4] found kaempferol and/or quercetin in 38 of 49 species; myricetin accompanied these flavonols in just four species, *C. aureus*, *C. candidus*, *C. olivieri* and *C. stellaris*. In our present survey of nine commercially available species (Table II), we found kaempferol glycosides in petals of all nine and quercetin glycosides in petals of seven. Two of the major flavonol glycosides in the petals were characterised as kaempferol 3-sophoroside and kaempferol 3-rutinoside-7-glucoside. The 3-sophoroside was found in petals of seven species and leaves of five (Table II). This glycoside is unusually acid-labile in solution, being converted to the aglucone and glucose even when standing at 4°. This is probably why Price *et al.* [11] were able to isolate free kaempferol in the flowers, since they left their extracts standing in the laboratory for 2–3 weeks. In fact, in direct extracts, all the flavonol is present in glycosidic form. Flavonols in fact do not appear to contribute to yellow flower colour in *Crocus*, since the glycosides occur in just as high a concentration in white flowered species as yellow. Derivatives of crocetin were found in the yellow flowered species, so that carotenoid is almost certainly the main source of yellow in this genus.

The 3-rutinoside-7-glucoside of kaempferol was isolated from petals of *C. chrysanthus*, *C. fleischeri*, *C. etruscus* and *C. minimus;* this substance was first isolated from *Equisetum palustris* [13], but is still a relatively uncommon plant glycoside. Several other disubstituted flavonol glycosides were noted, including kaempferol with 3,4′-disubstitution, and further work is needed on these floral pigments.

Cyanic colours (lilac, mauve and blue) in *Crocus* were reported to be delphinidin-based, on the basis of simple colour reactions [11, 14] and chromatographic studies have confirmed this view. Delphinidin 3,5-diglucoside was reported in *C. sativus* [15] and the same pigment has now been found in *C. laevigatus*. The other five cyanic species (Table II) also has simple delphinidin glycosides, with traces of petunidin derivatives. However, no malvidin has been reported in these flowers, although wider surveys are still needed to establish its absence.

Discussion

The discovery of 6-hydroxyflavones in *Crocus* (Iridaceae) brings the number of family records of these distinctive leaf constituents in the Monocotyledoneae [16–19] to five (Table III). They are more common in dicotyledonous groups occurring there in some twenty families, but especially in the highly specialised Bignoniaceae, Globulariaceae, Labiatae and Scrophulariaceae [20]. In the Dicotyledoneae, the synthetic capacity to insert an extra hydroxyl at the 6-position of the flavone nucleus seems to have arisen more than once in the course of angiosperm evolution. The same is true in the monocotyledons, since the five families (Table III) containing 6-hydroxyflavones are quite unrelated to each other. They are, however, all fairly 'advanced' groups in morphological terms, so that 6-hydroxylation may be regarded as a sign of chemical advancement, as it is in the Dicotyledoneae.

Within the five families containing 6-hydroxyflavones, it is interesting that the Orchidaceae are distinctive in having 6-O-methylation superimposed on 6-hydroxylation, while in Iridaceae (*Crocus*), there is additional 7-O-methylation. It will be interesting to see if surveys in progress will reveal further sources of these unusual flavonoid constituents among iridaceous plants. It may be of significance that isoflavones with 6-hydroxyl substitution, *e.g.* irigenin or 5,7,3′-trihydroxy-6,4′,5′-trimethoxyisoflavone, are well known to occur in *Iris*, which is in a different tribe from *Crocus*.

The present results establish a complex flavonoid pattern within those species of *Crocus* so far examined. Flavonols, in a range of glycosidic combinations, are the dominant type, with glycoflavones being regularly present. Flavones, on the other hand, as O-glycosides are more restricted and thus may have more potential as taxonomic markers in sectional classification. Detailed studies of the glycosidic variation of flavonoids in the genus should be systematically rewarding.

Experimental

Plant material

In the case of cultivated *Crocus* species, corms were purchased commercially and grown in a cool glasshouse, the leaves being collected after flowering. The wild *Crocus* species studied were kindly provided from the collection at the Royal Botanic Gardens, Kew.

Isolation of 6-Hydroxyflavones

The fresh leaf tissue was extracted with boiling 80% MeOH, the chlorophyll removed by washing with petroleum (b.p. 60–80°) and the concentrated extracts were separated by chromatography on 3 MM paper in 15% HOAc. Bands which appeared dark absorbing in UV light and unaffected by fuming with NH_3 vapour were eluted and the concentrated eluates separated on 3 MM paper in BAW. Two bands (R_f 18 and 36) were obtained from *C. chrysanthus* cv. 'Cream Beauty' to even-

Table III. Distribution of 6-hydroxyflavones within the Monocotyledoneae.

Super order and family	Genus	Aglycones present	Reference
Commeliniflorae			
Commelinaceae	*Setcreasia, Tradescantia*	6-hydroxyluteolin	[16]
Cyperaceae	*Lagenocarpus*	6-hydroxyluteolin	[17]
Liliiflorae			
Bromeliaceae	*Pitcairnia, Puya, Tillandsia*	6-hydroxyluteolin and scutellarein	[18]
Orchidaceae	*Oncidium, Eria, Odontoglossum*	scutellarein 6-methyl ether and 6,4′-dimethyl ether	[19]
Iridaceae	*Crocus*	6-hydroxyluteolin and its 7-methyl ether, scutellarein and its 7-methyl ether	this paper

Table IV. R_f data for 6-hydroxyflavone glycosides of *Crocus*.

Glycoside	R_f values (× 100) in			
	BAW	15% HOAc	H_2O	CAW
1	18	03	00	07
2	37	14	02	07
3	48	15	06	47
4	38	09	03	30
5	62	25	03	76

BAW = *n*-BuOH–HOAc–H_2O (4:1:5, top), CAW = $CHCl_3$–HOAc–H_2O (1:1:1, lower), all measurements from TLC on microcrystalline cellulose.

tually yield **1** and **4**; three bands (R_f 37, 48 and 60) were obtained from *C. minimus* to yield **2, 3** and **5**; and two bands (R_f 48 and 60) were obtained from *C. corsicus* to yield **3** and **5,** respectively. R_f data for the purified glycosides are collected in Table IV.

6-Hydroxyluteolin 7-glucoside **(1)** showed identical spectral and R_f properties to authentic material, isolated from *Catalpa bignonioides* leaf [21] and gave 6-hydroxyluteolin and glucose on hydrolysis. **1** co-chromatographed without separation with the authentic material in 4 solvents.

6-Hydroxyluteolin 7-rhamnosylglucoside (**2**) was identical to **1** in spectral properties with λ_{max} in MeOH at 287 and 349 nm, in MeOH + NaOAc 265i, 366; in MeOH + H_3BO_3 265, 364 nm. It gave equal amounts of glucose, rhamnose and 6-hydroxyluteolin on acid hydrolysis. R_f data (Table IV) were consistent with attachment at the 7-position of a rhamnosylglucose but the nature of the interglycosidic link could not be further determined.

6-Hydroxyluteolin 7-methyl ether 6-glucoside (**3**). This gave glucose and an aglucone, as a yellow powder, m.p. 245–6°, identified as 6-hydroxyluteolin 7-methyl ether on acid hydrolysis. The aglucone, on demethylation with pyridinium chloride, gave 6-hydroxyluteolin (identified by co-chromatography). That the aglucone was a monomethyl ether followed from the molecular ion on mass spectroscopy (found 316.0574 $C_{16}H_{12}O_7$ requires 316.0582). The location of the methyl group at position 7 followed from the UV spectral analysis: maxima in MeOH at 254, 273, 346; in MeOH + NaOAc 265, 275, 388; in MeOH + NaOAc/H_3BO_3 263, 376i, 380: in MeOH + $AlCl_3$ 275 and 425; in MeOH + $AlCl_3$/HCl 261, 279, 294i, 368; and in MeOH + NaOH 264 and 407 nm. The structure of the aglucone was confirmed by the mass spectral fragmentation: M^+ 316 (100%), M-CH_3 301.0344 ($C_{15}H_9O_7$ requires 301.0345) (77%), M–CO–H 287 (15%), M–CO–CH_3 273 (51%), tetraoxygenated A ring fragment 166.9973 ($C_7H_3O_5$ requires 166.9979) (36%), dioxygenated B-ring fragment 134.0370 ($C_8H_6O_2$ requires 134.0366) (53%). The identity of **3** as the 6-glucoside followed from R_f properties indicating monoglucosylation and from its spectral properties: maxima in MeOH 258, 271, 348; + NaOAc 267, 368; + NaOAc/H_3BO_3 264, 375; + NaOH 277, 410; and + $AlCl_3$ 275, 418 nm.

Scutellarein 7-glucoside **(4)** gave scutellarein and glucose on acid hydrolysis and was clearly a monoglucoside from R_f data (Table IV). Its formation as the 7-glucoside followed from its spectral properties, which were identical to authentic scutellarein 7-glucoside [22].

Scutellarein 7-methyl ether 6-glucoside **(5).** The structure of this pigment followed from similar evidence adduced in the case of **3.** The scutellarein 7-methyl ether released on acid hydrolysis had UV maxima in MeOH at 277 and 333 nm and displayed no shifts with NaOAc or NaOAc/H_3BO_3. MS data: M^+ 300 (100%), M–CH_3 285 (69%), M–CO–H 271 (11%), M–CO–CH_3 257 (67%), tetraoxygenated A-ring 167 (29%), monooxygenated B-ring fragment 118 (44%).

Acacetin was isolated by paper chromatography from acid-hydrolysed extracts of *C. laevigatus.* It was identical in spectral and R_f properties to an authentic sample, prepared by acid hydrolysis of linarin, from *Linaria vulgaris* flowers [6]. On demethylation with HBr/HOAc, it gave apigenin.

Mangiferin was isolated in crystalline form (m.p. 270–2°) from leaves of *C. aureus.* It was identical to authentic material from *Iris* flowers [23] by m.m.p., spectral analysis and co-chromatography.

Kaempferol 3-sophoroside. Its presence/absence in leaves and petals of *Crocus* ssp. (Table II) was determined by 2-dimensional PC in BAW and 5% HOAc. It was isolated in quantity from flowers of *C. laevigatus* and *C. korolkowii* and was identified by standard procedures [6] as the 3-sophoroside. This was confirmed by co-chromatography in 6 solvents with a synthetic sample kindly supplied by Professor H. Wagner.

Kaempferol 3-rutinoside-7-glucoside was isolated from petals of *C. fleischeri* and *C. etruscus* and then

identified by standard procedures [6]. In particular, its structure followed from the evidence of mild acid hydrolysis to yield kaempferol 7-glucoside and β-glucosidase hydrolysis to yield kaempferol 3-rutinoside.

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